REMARKS

A. Summary of the Invention

Broadly, the present invention relates to a method of determining the identity of one or more nucleotide bases at a plurality of specific positions in one or more nucleic acid molecules of interest. The method includes the step of treating a sample comprising the nucleic acid molecules of interest if the nucleic acid molecules are double stranded so as to obtained unpaired nucleotide bases spanning the specific positions. Alternatively, a sample of the nucleic acid of interest may be used directly if the nucleic acid molecules are single stranded. The method of the invention further includes the step of contacting the sample with a plurality of different oligonucleotide primers. Each different oligonucleotide primer hybridizes to a corresponding different stretch of nucleotide bases present in the nucleic acid molecules of interest which is immediately adjacent to the specific position of a nucleotide base to be identified with that oligonucleotide primer, so as to form a duplex such that the nucleotide base to be identified is the first unpaired base of the nucleic acid molecule of interest immediately downstream of the 3' end of the primer. Each different oligonucleotide primer comprises a corresponding different affinity moiety. The oligonucleotide primer comprising the affinity moiety is capable of hybridizing with a nucleic acid template and undergoing a nucleic acid template-dependent primer extension reaction with terminators of a terminator reagent. The affinity moiety permits affinity separation of the extended oligonucleotide primer from the terminator reagent. The method of the invention comprises the further step of contacting the duplexes with a terminator reagent which includes four different terminators of a nucleic acid template-dependent primer extension reaction. The terminator reagent is free of dATP, dCTP, dGTP, and dTTP. Each terminator comprises a different detectable label corresponding to the terminator. One of the terminators is complementary to a nucleotide base to be identified by each of the oligonucleotide primers. The contacting is carried out in a primer extension reaction medium under conditions sufficient to permit a template dependent primer extension reaction, which incorporates the complimentary terminator onto the 3' end of each of the different oligonucleotide primers to thereby extend the 3' end of each of the primers by one terminator. The method of the invention further includes the

step of affinity separating the respective extended oligonucleotide primers from the primer extension reaction medium by causing each of the extended oligonucleotide primers to contact an affinity group attached to a solid support. The affinity group is complementary to the affinity moiety incorporated in the oligonucleotide primer. Finally, the method of the invention includes the step of determining the presence and identity of the nucleotide base at each of the respective specific positions in the one or more nucleic acid molecules of interest by detecting the detectable label of the terminator incorporated at the 3' end of each of the affinity separated extended oligonucleotide primers.

B. Summary of the Outstanding Office Action

Claims 64, 66, 67, and 69 through 70 inclusive were rejected in the Office Action of 5 September 2006 under 35 U.S.C. § 103(a) as unpatentable over European published patent application EP 0 412 883 A1 to Cohen *et al.* ("the Cohen *et al.* '883 published European application") or French patent 2,650,840 also to Cohen *et al.* ("the Cohen *et al.* '840 French patent"), each in view of international PCT published patent application WO 90/11372 to Davis *et al.* ("the Davis *et al.* '372 PCT published application").

It was noted in the Office Action that the Cohen *et al.* '883 published European application claimed priority to a French patent application 8910802, which issued as the '840 French patent. Since both the Cohen *et al.* '883 published European application and the Cohen *et al.* '840 French patent are in the French language and since an English translation of the '840 patent has been provided in the present case, only the '840 French patent as translated will be referred to specifically in the discussion which follows.

It was asserted in the outstanding Office Action that the '840 French patent disclosed a method of determining the identity of one or more nucleotide bases in a nucleic acid molecule which involved contacting a single-stranded nucleic acid sample with an oligonucleotide primer to form a duplex between the primer and complementary target nucleic acids present in the sample, wherein the primer hybridized immediately 3' of the nucleotide to be determined. It was asserted that the method of the '840 French patent further included the step of contacting the

duplexes with a solution containing four different terminators, each labeled with a different detectable moiety. The method of the '840 French patent assertedly further included the steps of extending the primer with the terminator and determining the identity of the incorporated terminator to determine the identity of the nucleotide base. It was conceded in the Office Action of 5 September 2006 that the Cohen *et al.* '840 French patent did not disclose performing the primer extension reaction using multiple primers, each comprising a different affinity moiety.

It was asserted in the Office Action of 5 September 2006 that the Davis *et al.* '372 PCT published application disclosed a method for determining the identity of one or more nucleotide bases in a nucleic acid molecule which comprised contacting a single-stranded nucleic acid molecule with an oligonucleotide primer to form a duplex between the primer and complementary target nucleic acids. It was asserted that the duplexes were contacted with a solution containing labeled dNTPs to extend the primer with the dNTPs, assertedly such that if the primer were perfectly complementary with the target nucleic acid, an extension product would be formed, but if the primer contained a mismatch at or near the 3' end of the primer, an extension product would not be formed. It was asserted in the outstanding Office Action that, in the method of the '372 PCT published application, the presence of an extension product was detected in order to determine the identity of a nucleotide base. In the Office Action, it was asserted that the '372 PCT published application disclosed that the identity of multiple nucleotides could be determined simultaneously by using a mixture of different oligonucleotides, in which each oligonucleotide comprised a unique tail. It was asserted that, following the extension reaction, the primer extension/target nucleic acid complex was denatured and the primer extension product was hybridized to a solid support having bound thereto sequences complementary to the primer tail. It was asserted in the Office Action of 5 September 2006 that the unique tail allowed for the primers to be immobilized at specific locations on the support.

It was asserted in the Office Action of 5 September 2006 that it would have been obvious to have modified the method of the Cohen *et al.* '840 French patent so as to have used multiple primers, each having a different tail, and to have separated the primer extension products from the reaction medium by contacting the extension products with a solid support having

immobilized thereon nucleic acid with a sequence complementary to the tail sequence – referred to in the Office Action as a "capture probe" – assertedly in order to accomplish objectives assertedly set forth in the Davis *et al.* '372 PCT published application.

It was noted in the outstanding Office Action that a rejection of claims of the subject application as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application had been traversed in an earlier reply filed on 26 June 2006 on behalf of the applicants on the grounds that the Cohen *et al.* French patent taught away from any technique that required the immobilization of a nucleic acid on a membrane. It was pointed out in the 26 June 2006 reply that the Cohen *et al.* '840 French patent drew no distinction between reversibly or irreversibly immobilizing nucleic acids on a membrane, but taught that the method of the patent had the general advantage of not requiring immobilization of a nucleic acid on a membrane. In the outstanding Office Action, it was noted that it had been observed in the prior reply that the Cohen *et al.* '840 French patent taught that immobilization of a nucleic acid on a membrane was a disadvantage of both the method of Southern blotting and the method of United States patent No. 4,656,127 to Mundy ("the Mundy '127 patent") and that it had been reasoned that a person of ordinary skill in the art would have recognized that the method of the Davis *et al.* '372 PCT published application, which involved spotting an oligonucleotide onto a substrate, shared the disadvantages of previously known techniques requiring immobilization on a membrane. It was observed in the Office Action of 5 September 2006 that it had been concluded in the prior reply of 26 June 2006 that the Cohen *et al.* '840 French patent taught away from the combination of the Cohen *et al.* '840 French patent with the Davis *et al.* '372 PCT published application.

In the outstanding Office Action of 5 September 2006, it was asserted that the Cohen *et al.* '840 French patent would not have taught away from using the method of the Davis *et al.* '372 PCT publication notwithstanding a conceded teaching of the French patent "away from irreversible immobilization of a target nucleic acid prior to probe hybridization." [Underlining and emphasis omitted.] It was asserted in the Office Action that the method of the Davis *et al.* publication was distinct from the method of the Mundy '127 patent and the method of Southern,

without any indication of which of the short-probe Southern-blot method or the long-probe Southern-blot method referred to in the Cohen *et al.* '840 French patent was asserted to be distinct. It was asserted that in the method of the Davis *et al.* publication hybridization of a target nucleic acid would occur in solution and primer extension products would be formed in solution. According to the outstanding Office Action, subsequent reversible immobilization of the primer extension products on a solid support in the method of the Davis *et al.* publication would not interfere with the specificity of the detection process assertedly because the specificity of the detection process would have occurred at the steps of primer hybridization and primer extension, both carried out in solution. In the 5 September 2006 Office Action, the contention in the prior reply of 26 June 2006 that the method of the Davis *et al.* '372 PCT publication involved irreversibly binding tail-complementary oligonucleotides to a substrate was noted, but it was asserted that the disclosure of the publication relating to binding tail-complementary oligonucleotides to a substrate was "not directed to the immobilization of a target nucleic acid, particularly prior to hybridization of a target nucleic acid to a primer or probe, or prior to primer extension." [Emphasis omitted.] It was asserted in the outstanding Office Action that hybridization of extension products to the immobilized tail-complementary oligonucleotides in the method of the Davis *et al.* publication "[did] not require the specificity that is relied upon to detect the presence of a single nucleotide variation between a target nucleic acid and a probe."

Claim 68 was rejected in the Office Action of 5 September 2006 under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '883 published European application or the Cohen *et al.* '840 French patent, each in view of the Davis *et al.* '372 PCT published application and United States patent No. 5,332,666 to Prober *et al.* ("the Prober *et al.* '666 patent"). It was conceded in the outstanding Office Action that the hypothetical combination of the Cohen *et al.* patent and the Davis *et al.* published application proposed in the Office Action did not disclose using a terminator that comprised arabinoside triphosphate. It was asserted that the Prober *et al.* '666 patent disclosed that a terminator may contain an arabinose as the sugar group. It was asserted in the 5 September 2006 Office Action that it would have been obvious to one of ordinary skill in the art to have modified the method of

the Cohen *et al.* '840 French patent so as to have a terminator comprising an arabinoside triphosphate.

It was asserted in the Office Action that the comments with respect to the position taken on behalf of the applicants in the previous reply of 26 June 2006 applied equally to the position taken on behalf of the applicants in that previous reply with respect to the rejection of claim 68.

Claim 71 was rejected in the outstanding Office Action under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '883 published European application or the Cohen *et al.* '840 French patent, each in view of the Davis *et al.* '372 PCT published application and United States patent No. 4,962,020 to Tabor *et al.* ("the Tabor *et al.* '020 patent"). In the Office Action it was stated that the hypothetical combination of the Cohen *et al.* '840 French patent and the Davis *et al.* '372 PCT published application proposed in the Office Action did not disclose including pyrophosphatase in the primer extension medium. It was asserted that the Tabor *et al.* '020 patent disclosed including pyrophosphatase in primer extension reactions to remove pyrophosphate which builds up in such reactions. The Tabor *et al.* '020 patent assertedly disclosed that, in the presence of pyrophosphate, DNA polymerase adds pyrophosphate to the 3' terminal nucleotide, assertedly causing release of dideoxynucleoside 5'-triphosphates, removing the block at the 3' terminus. It was asserted that in the Office Action of 5 September 2006 that it would have been obvious to one of ordinary skill in the art to have modified the method of the Cohen *et al.* '840 French patent so as to have included pyrophosphatase in the reaction medium assertedly to eliminate pyrophosphorolysis activity of DNA polymerase assertedly to reduce the probability that a labeled terminator would be removed and unlabeled dideoxynucleotides would be released into the reaction medium.

It was asserted in the Office Action that the comments with respect to the position taken on behalf of the applicants in the previous reply of 26 June 2006 applied equally to the position taken on behalf of the applicants in that previous reply with respect to the rejection of claim 71.

C. Request for Reconsideration

Reconsideration of the subject application in light of the comments below is respectfully requested.

D. The Rejections Under 35 U.S.C. § 103(a)

D.1 The Cohen *et al.* '840 French Patent in View of the Davis *et al.* '372 PCT Published Application

The attorneys for the applicants stand by the position explained in the earlier reply filed on 26 June 2006 that the Cohen *et al.* '840 French patent taught directly away from the combination, proposed in Office Action rejections under 35 U.S.C. § 103(a), of the process for identifying a single base in a nucleic acid sequence of the Cohen *et al.* patent with the method of the Davis *et al.* '372 PCT published application for testing a single sample of DNA simultaneously for multiple alleles or for testing simultaneously at multiple loci for a single allele or multiple alleles.

In this regard, we point out again that the Cohen *et al.* '840 French patent expressly distinguished the method of the patent from three previously known techniques for identifying a mutation in nucleic acid involving a single nucleotide position: (1) a long-probe Southern blot technique, (2) a short-probe technique which it is submitted that persons of ordinary skill in the art would have recognized to be a type of Southern-blot technique, and (3) the method of the Mundy '127 patent. The remarks in the 5 September 2006 Office Action responding to the position that the Cohen *et al.* '840 French patent taught against the proposed combination of the process of the Cohen *et al.* patent with the method of the Davis *et al.* '372 PCT published application referred to only two of the three previously known techniques expressly distinguished from the method of the Cohen *et al.* patent in the patent specification – specifically, the technique referred to in the Cohen *et al.* patent as the short-probe Southern blot technique and the method of the Mundy '127 patent. We contend that the position in the outstanding Office Action on whether the Cohen *et al.* French patent taught against the method of the Davis *et al.* '372 PCT publication is substantially undercut by the express teachings of the Cohen *et al.*

'840 French patent against the version of the Southern-blot technique which did not involve allele-specific hybridization; namely, the long-probe Southern-blot technique; which was not addressed in the Office Action.

As noted in the previous reply of 26 June 2006, persons of ordinary skill in the art would have understood the long-probe Southern blot technique described in the Cohen *et al.* '840 French patent to be a conventional restriction-site analysis method of which the method described in E. M. Southern, *Journal of Molecular Biology*, volume 98, pages 503 through 517 (1975) ("the Southern publication") was illustrative. A copy of the Southern publication was submitted with the previous reply. In the long-probe Southern-blot technique as applied to identifying a single nucleotide polymorphism in DNA, the polymorphism was identified by whether or not a particular restriction enzyme cut the DNA at the site of the polymorphism. Application of the restriction enzyme to the DNA was carried out in solution prior to size separation of the enzyme-treated DNA by electrophoresis, immobilization of the size-separated enzyme-treated DNA in single-strand form on a membrane, and hybridization of a labeled nucleic-acid "long probe" to the immobilized strands of the DNA to identify the physical positions on the membrane, and hence the size, of any DNA strands complementary to the probe.

The textbook *Biochemistry*, third edition, by L. Stryer at page 169 ("the Stryer textbook") described analysis of DNA for the presence of the sickle-cell gene, which was a known single-nucleotide polymorphism, using a restriction enzyme which recognized the sequence in the region of the polymorphism. A copy of page 169 of the Stryer textbook is being submitted with the present reply. Significantly with respect to the attempts made in the outstanding Office Action to draw distinctions between, on the one hand, immobilization of nucleic acid in the method of the Davis *et al.* '372 PCT published application and, on the other hand, immobilization of nucleic acid in the short-probe Southern blot technique and in the method of the Mundy '127 patent, the technique for analyzing for the presence of the sickle-cell gene described on page 169 of the Stryer textbook entailed the step of detecting the single-nucleotide polymorphism by digestion of DNA with a restriction enzyme, which persons of ordinary skill in the art would have recognized was to be carried out on DNA in solution, not on DNA

immobilized on a membrane. According to lines 12 through 15 of page 169 of the Stryer textbook, following the digestion by the restriction enzyme, "[t]he fragments in the digested sample of DNA are separated by gel electrophoresis and visualized by Southern blotting with a ^{32}P-labeled probe that is complementary to [a] 1.1kb fragment" present in both the normal and the sickle-cell gene. [Citation omitted.] Thus, as persons of ordinary skill in the art would have appreciated, the labeled probe used to visualize the electrophoresis pattern from the digested DNA in the gene-analysis technique described on page 169 of the Stryer textbook did not itself serve to discriminate between the two single-nucleotide polymorphisms detected by the technique, but merely marked the location of size-separated fragments on a membrane to which fragments such probes were hybridized according to the Southern blot technique.

The Cohen *et al.* '840 French patent specifically described a Southern-blot technique involving "long probes" in terms which persons of ordinary skill in the art would have recognized applied to the gene-analysis technique described on page 169 of the Stryer textbook discussed above. The Cohen *et al.* French patent also described a short-probe technique which, it is submitted, persons of ordinary skill in the art would have recognized to be a type of Southern-blot technique different from the long-probe Southern-blot technique. The disclosure of the Cohen *et al.* '840 French patent at page 2, line 19 through page 3, line 17 concerning the long-probe and short-probe Southern-blot techniques and certain disadvantages of the two techniques is set out below:

> Thus, to detect a mutation involving a single base, depending on the case generally two types of probes can be used: nucleic acid probes called long probes, generally over 150 nucleotides, or nucleic acid probes called short probes, generally between 17 and 24 nucleotides. If the mutation occurs at a site recognized specifically by an enzyme called a restriction enzyme, the Southern blot technique can be used. This technique includes stages of isolating the DNA, digestion by the restriction enzyme, electrophoresis on gel, transfer onto a membrane, and hybridization by means of a long probe involving the region of the mutation; after washing and autoradiography, analysis of the size of the fragments obtained permits confirmation or invalidation of the presence of the mutation. This very cumbersome process requires that the mutation involve a restriction site. If this is not the case, a short nucleotide probe of 17 to 24 nucleotides can be synthesized, the center of which coincides with the mutation that one wishes to

> detect. By selecting suitable hybridization and rinsing conditions (specific for each system), hybridization by means of marked oligonucleotides can be achieved only in case of perfect equivalence (the difference of a single nucleotide, particularly at the site of the mutation, results in destabilization of the hybridization).
>
> However, these various methods all have a certain number of disadvantages:
>
> – the temperature conditions are difficult to master to achieve suitable hybridization;
>
> – the mandatory presence of a restriction site may be required;
>
> – the nucleic acid is immobilized on a membrane (Southern blot).

In the preceding quotation concerning certain prior methods for detecting a mutation involving a single base and disadvantages of such methods, the Cohen *et al.* '840 French patent identified three separate disadvantages and introduced the three disadvantages with the following language: "these various methods <u>all have</u> a certain number of disadvantages." [Underlining added.] The first and third disadvantages were stated unconditionally in the Cohen *et al.* French patent; the second disadvantage was stated as a possibility; i.e. "a restriction site *may* be required." [Emphasis added.]

In the first paragraph of the quotation bridging pages 2 and 3 of the Cohen *et al.* '840 French patent set out in the preceding paragraph, it was noted that the process involving hybridization by means of a long probe required that the mutation involve a restriction site, and that, if that were not the case, a process involving hybridization by means of a short probe could be used. In view of the first paragraph of the quotation, the second disadvantage quoted above stating that "the mandatory presence of a restriction site may be required" would have been understood as applying to the long-probe Southern-blot technique, but not to the short-probe Southern-blot technique as described.

In contrast, the first and third disadvantages identified in the quotation bridging pages 2 and 3 of the Cohen *et al.* '840 French patent set forth above would have been understood by persons of ordinary skill in the art as applying both to the long-probe Southern-blot technique and to the short-probe Southern-blot technique as described, particularly since the two disadvantages were stated in unconditional terms. Thus it is submitted that the Cohen *et al.*

French patent taught that the long-probe Southern-blot technique shared with the short-probe Southern-blot technique a pair of disadvantages; namely, "the temperature conditions are difficult to master to achieve suitable hybridization" and "the nucleic acid is immobilized on a membrane (Southern blot)." Moreover, it is submitted that persons of ordinary skill in the art would have understood from the Cohen *et al.* French patent that the long-probe Southern-blot technique had the two disadvantages of: "the temperature conditions are difficult to master to achieve suitable hybridization" and "the nucleic acid is immobilized on a membrane (Southern blot);" notwithstanding the recognition of such persons, as discussed above in connection with the gene-analysis technique described on page 169 of the Stryer textbook and consistent with the description of the long-probe Southern-blot technique in the Cohen *et al.* French patent, that the probe of the long-probe Southern-blot technique was used to visualize an electrophoresis pattern from DNA digested by a restriction enzyme in solution and did not discriminate between the two single-nucleotide polymorphisms, but merely marked the location of size-separated fragments on a membrane to which fragments such probes were hybridized according to the Southern blot technique.

We submit further that the disadvantage of temperature conditions being difficult to master to achieve suitable hybridization and the disadvantage of requiring nucleic acid to be immobilized on a membrane identified by the Cohen *et al.* '840 French patent for the long-probe Southern-blot technique would have been recognized by persons of ordinary skill in the art to apply essentially equally to the method of the Davis *et al.* '372 PCT publication. The method of the Davis *et al.* publication involved irreversibly binding tail-complementary oligonucleotides to a membrane or other substrate at specific locations corresponding to the respective tail sequences of a set of tail-bearing extension primers and applying putative extension products to the substrate under conditions permitting hybridization of the tails of the extension products to respective oligonucleotides complementary to the tails bound to the substrate at specific locations for identifying each location having extension product which bore a label hybridized to the tail-complementary oligonucleotide bound to the substrate at the specific location and so identifying a particular single-nucleotide-polymorphism allele corresponding to the location. As indicated in the Office Action of 5 September 2006, the method of the Davis *et al.* '372 PCT publication did

not require hybridization specificity sufficient to detect the presence of a single nucleotide variation between a target nucleic acid and a probe. However, the long-probe Southern-blot technique likewise did not require such hybridization specificity, yet the Cohen *et al.* '840 French patent disclosed that in the long-probe Southern-blot technique, "the temperature conditions are difficult to master to achieve suitable hybridization," which the Cohen *et al.* patent expressly characterized as a disadvantage of the technique. Persons of ordinary skill in the art with the Cohen *et al.* patent and the Davis *et al.* publication at hand would have recognized that in the method of the Davis *et al.* publication, the temperature conditions would have been difficult to master to achieve suitable hybridization just as in the long-probe Southern-blot technique, which would have been a disadvantage leading away from the hypothetical combination of the process of the Cohen *et al.* patent with the method of the Davis *et al.* publication proposed by the examiner in the Office Action of 5 September 2006.

Additionally, the Cohen *et al.* '840 French patent distinguished both the long-probe Southern blot technique and the short-probe Southern blot technique from the method of the patent not only on the basis of temperature conditions being difficult to master to achieve suitable hybridization, but also on the basis of a requirement – specifically characterized in the Cohen *et al.* patent as a disadvantage – to immobilize nucleic acid on a membrane in Southern blot techniques generally. The requirement to immobilize nucleic acid on a membrane was a distinguishing feature shared in common by the three previously known techniques distinguished from the method of the Cohen *et al.* patent in the patent specification. In teaching that it was a disadvantage to immobilize nucleic acid on a membrane in the long-probe Southern blot technique, the short-probe Southern blot technique and the method of the Mundy '127 patent, it is submitted that the Cohen *et al.* '840 French patent taught directly away from the combination of the process of the Cohen *et al.* patent with the method of the Davis *et al.* '372 PCT published application proposed in Office Action of 5 September 2006, since the method of the Davis *et al.* published application involved immobilizing nucleic acid on a membrane.

At page 6, lines 29 through 33 of the Cohen *et al.* '840 French patent, it was disclosed that a purported advantage of the process of the patent was that the process did not require

immobilization of the nucleic acid on a membrane. As may be seen, for example, at page 1, lines 5 through 13, and page 2, lines 8 through 18 of the Cohen *et al.* patent, in the context of the patent, the term "nucleic acid" applied generally to each strand of hybridized DNA or RNA, including probes 150 nucleotides long and shorter probes. Furthermore, at page 3, lines 23 through 29 of the Cohen *et al.* patent, the term "nucleic acid" was applied to a double-stranded hybrid. As noted above, the necessity to immobilize nucleic acid on a membrane was specifically pointed out in the Cohen *et al.* '840 French patent to be a disadvantage shared in common by the previously-known Southern blot technique generally – described in the Cohen *et al.* patent as including two substantially different variants: a long-probe variant and a short-probe variant – and the substantially different method of the Mundy '127 patent. See page 3, lines 10 through 17 and page 4, lines 14 through 17 of the Cohen *et al.* patent. It is submitted therefore that the Cohen *et al.* '840 French patent would have directly led persons skilled in the art away from any technique which shared the requirement of immobilization of nucleic acid on a membrane in either single-stranded or double-stranded hybrid form.

Persons of ordinary skill in the art would have recognized, it is submitted, that the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application was just such a technique involving immobilization of nucleic acid on a membrane from which the Cohen *et al.* patent taught away. The Davis *et al.* published application disclosed a technique for determining the existence or nonexistence of a test nucleotide on a strand of DNA which employed a polymerization agent capable of synthesizing an extension product if there were a match between the test nucleotide on the DNA strand and a nucleotide opposite on an extension primer, but not if there were a mismatch. According to page 5, line 19 through page 6, line 22 of the Davis *et al.* '372 PCT published application, a single sample of DNA could be tested simultaneously for multiple alleles at a single locus or for a single allele or multiple alleles at multiple loci by treating the DNA with a plurality of different oligonucleotide primers, each primer being complementary to a different allele and each having a unique oligonucleotide "tail." The primers and the DNA were then subjected to conditions that would have allowed the primers and DNA to pair and labeled extension products to form if there were a match between a test nucleotide and the opposite nucleotide on the primer, but not if there were a mismatch. It

was disclosed at page 6, lines 7 through 22 and page 21, lines 8 through 12 of the Davis *et al.* published application that the presence or absence of a particular extension product could be determined by applying the putative extension products to a substrate such as filter paper, nylon, or nitrocellulose "spotted" at distinct locations with unique oligonucleotides complementary to each of the unique oligonucleotide tails. The Davis *et al.* '372 PCT published application at page 8, lines 1 through 5, for example, that such spotting of oligonucleotides complementary to each of the unique oligonucleotide tails on the substrate preferably resulted in the oligonucleotides "being firmly bound to the substrate but accessible for hybridization with complementary sequences." According to the Davis *et al.* published application, if a particular extension product existed, it would have attached to the substrate at only one location by way of hybridization of the unique tail to the complementary oligonucleotide found only at that location on the substrate. The Davis *et al.* '372 PCT published application disclosed that by detecting the presence of a labeled extension product hybridized to a complementary oligonucleotide bound to the substrate at a specific location, the presence or absence of a specific allele in the test DNA could be determined.

The Cohen *et al.* '840 French patent drew no distinction between reversibly immobilizing nucleic acid on a membrane and irreversibly immobilizing nucleic acid on a membrane, but declared without qualification on page 6, lines 29 through 33 of the specification that an advantage of the process of the patent was that the process did not require immobilization of the nucleic acid on a membrane. As pointed out above, the term "nucleic acid" was used in the Cohen *et al.* French patent to refer to double-stranded hybrids and to single strands of nucleotides. Each of the three previously known techniques for identifying a single nucleotide mutation distinguished in the Cohen *et al.* patent from the process of the patent in terms of having the disadvantage of requiring nucleic acid immobilized on a membrane would, it is submitted, have been expected by persons of ordinary skill in the art generally to have involved detecting a hybrid nucleic-acid complex immobilized on a membrane, in which a first component of the hybrid complex was a nucleic acid effectively irreversibly bound to the membrane and a second component of the hybrid complex was a labeled oligonucleotide of some sort reversibly bound to the first component by hybridization. Moreover, persons of ordinary skill in the art

would have recognized, it is submitted, that the method of the Davis *et al.* '372 PCT published application likewise involved detecting a hybrid nucleic-acid complex immobilized on a membrane in which a first component of the hybrid complex was a nucleic acid effectively irreversibly bound to the membrane and a second component of the hybrid complex was a labeled oligonucleotide reversibly bound to the first component by hybridization and therefore such persons would have appreciated that the method of the Davis *et al.* published application necessarily entailed a feature which the Cohen *et al.* '840 French patent characterized specifically as a disadvantage not shared by the process disclosed in the Cohen *et al.* patent. Persons of ordinary skill in the art would thus have deemed it in no way obvious to combine the process of the Cohen *et al.* patent with the method of the Davis *et al.* '372 PCT published application as proposed in the Office Action of 5 September 2006.

For the reasons set forth above, it is submitted that, assuming for the sake of argument only that the hypothetical combination of the single-base-identification process of the Cohen *et al.* '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the outstanding Office Action would even have occurred to a person of ordinary skill on the art as of the effective date of the subject application, such a person would have recognized that the method of the Davis *et al.* '372 PCT published application involved immobilizing nucleic acid on a substrate and that the method would therefore have effectively shared the disadvantage of three previously known methods requiring immobilizing nucleic acid on a membrane specifically pointed out in the Cohen *et al.* patent and would have recognized further that the method of the Davis *et al.* '372 PCT published application would have shared the disadvantage of temperature conditions being difficult to master to achieve suitable hybridization pointed out in the Cohen *et al.* French patent for the long-probe Southern blot technique. It is submitted therefore that a person of ordinary skill in the art would not have attempted to combine the single-base-identification process of the Cohen *et al.* '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application in view of the teachings in the Cohen *et al.* patent directly away from such the hypothetical combination.

For the reasons set forth above, it is submitted that the rejection in the Office Action of 5 September 2006 of claims 64, 66, 67, and 60 through 70 inclusive of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application was without justification and should be withdrawn.

D.2 The Cohen *et al.* '840 French Patent in View of the Davis *et al.* '372 PCT Published Application and the Prober *et al.* '666 Patent

The Prober '666 patent in no way overcomes the teachings of the Cohen *et al.* '840 French patent against the hypothetical combination of the single-base-identification process of the '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the outstanding Office Action discussed in the preceding subsection and consequently the reasoning of the preceding subsection applies equally with respect to the rejection of claim 68 in the Office Action of 5 September 2006 under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Prober *et al.* '666 patent. It is submitted that the rejection in the Office Action of 5 September 2006 of claim 68 of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Prober *et al.* '666 patent was unjustified and should be withdrawn.

D.3 The Cohen *et al.* '840 French Patent in View of the Davis *et al.* '372 PCT Published Application and the Tabor *et al.* '020 Patent

As in the case of the Prober *et al.* '666 patent discussed in the preceding subsection, the Tabor *et al.* '020 patent in no way overcomes the teachings of the Cohen *et al.* '840 French patent against the hypothetical combination of the single-base-identification process of the '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the Office Action of 5 September 2006 discussed above and consequently the reasoning of the preceding subsection applies equally with respect to

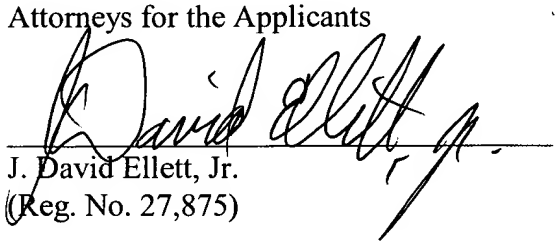
the rejection of claim 71 in the outstanding Office Action under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Tabor *et al.* '020 patent. It is submitted that the rejection in the Office Action of 5 September 2006 of claim 71 of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Tabor *et al.* '020 patent was unwarranted and should be withdrawn.

E. Conclusion

For the reasons set forth above, it is submitted that each of the claims of the subject application is allowable over the art of record considered alone or in any combination. Withdrawal of the rejections of the claims and allowance of the application is therefore earnestly solicited.

Respectfully submitted,

Attorneys for the Applicants

by: 

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